Ultrashort self-assembling peptidomimetic nanomaterials target resistant pathogenic infections

Laverty, G. (2015). *Ultrashort self-assembling peptidomimetic nanomaterials target resistant pathogenic infections*. Paper presented at IMAP 2015: 5th International Meeting on Antimicrobial Peptides, London , United Kingdom. http://peptideconferences.org/imap-2015

Ultrashort Self-assembling Peptidomimetic Nanomaterials Target Resistant Pathogenic Infections

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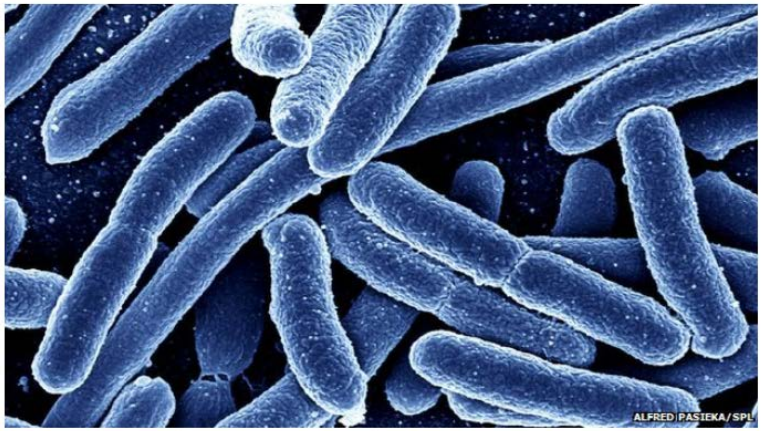
Queen's University Belfast

Antimicrobial Resistance

BBC NEWS

Superbugs to kill 'more than cancer' by 2050

COMMENTS (565)



Drug resistant E.coli bacteria are already a significant problem in Europe

Drug resistant infections will kill an extra 10 million people a year worldwide - more than currently die from cancer - by 2050 unless action is taken, a study says.

They are currently implicated in 700,000 deaths each year.

Related Stories

Analysis: Antibiotic apocalypse

sky NEWS

- Medical device related infections
- Increased reservoir of “superbugs”
- Persistent burden on:
  - Patient morbidity& mortality
  - Family and carers
  - Healthcare budgets

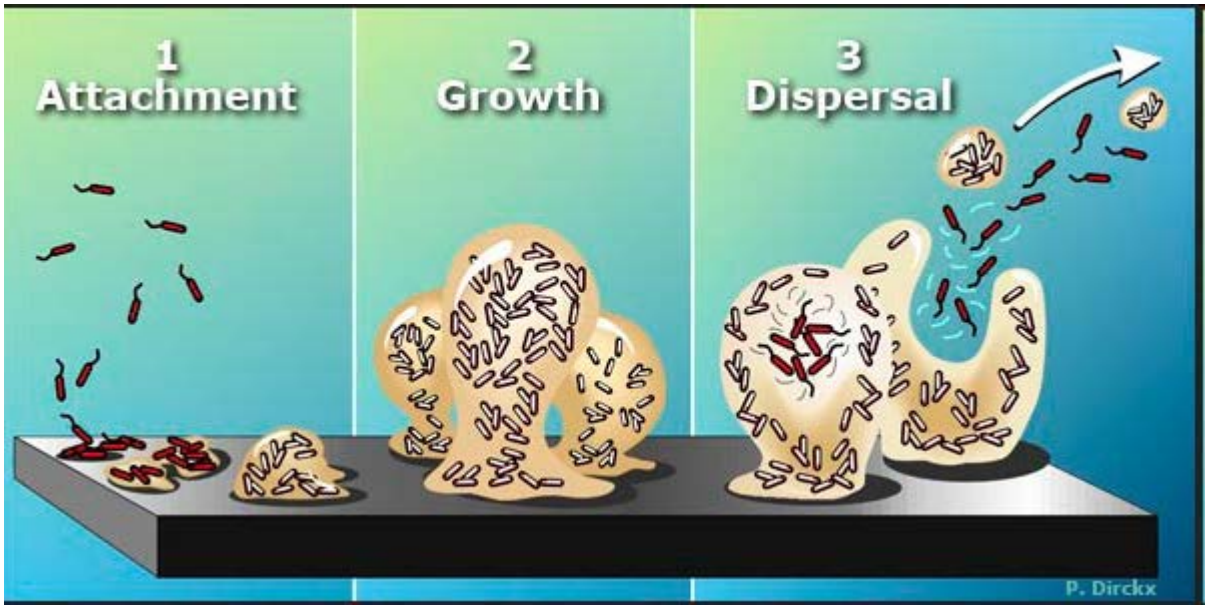
Superbugs 'Could Send UK Back To The Dark Ages'

Action is needed to stop the world entering a post-antibiotic era in which common infections and injuries can kill, say experts.

17:49, UK,
Wednesday 02 July 2014

Planktonic vs. Biofilm Bacteria

- Planktonic form: Free floating in liquid
- Biofilm form: sessile, composed of aggregated microcolonies of cells surrounded by a protective extracellular polymeric matrix
- Mature biofilms can resist 10-1000 times the concentrations of standard antibiotic regimens that are required to kill genetically equivalent planktonic forms



P. Dirckx, Centre for Biofilm Engineering, Montana State University, Bozeman

Biofilms and Implant-Associated Infections. Laverty, G., Gorman, S.P. and Gilmore, B.F. In: Biomaterials and Medical Device Associated Infections. Woodhead Publishing Ltd. 2014.

Current AMP Interests

Chem Biol Drug Des 2010; **75:** 563–569
Research Article

doi: 10.1111/j.1747-0285.2010.00973.x

Antimicrobial Activity of Short, Synthetic Cationic Lipopeptides

Garry Laverty, Martin McLaughlin, Christopher Shaw, Sean P. Gorman and Brendan F. Gilmore*

for new antimicrobial agents with activity against pathogens that are resistant to the available armoury of antibiotics (3,4).

BioMACROMOLECULES
Article
pubs.acs.org/Biomac

Ultrashort Cationic Naphthalene-Derived Self-Assembled Peptides as Antimicrobial Nanomaterials

Garry Laverty,*[,†] Alice P. McCloskey,[†,§] Brendan F. Gilmore,[†,§] David S. Jones,[†,§] Jie Zhou,[‡,§] and Bing Xu[‡,§]

Society For Biomaterials

Antimicrobial peptide incorporated poly(2-hydroxyethyl methacrylate) hydrogels for the prevention of *Staphylococcus epidermidis*-associated biomaterial infections

Garry Laverty, Sean P. Gorman, Brendan F. Gilmore

Chem Biol Drug Des 2014
Research Letter

Biofilm Eradication Kinetics of the Ultrashort Lipopeptide C_{12}-OOWW-NH_2 Utilizing a Modified MBEC Assay™

Garry Laverty*, Sean P. Gorman and Brendan F. Gilmore

Cationic antimicrobial peptides exist throughout the nature as defense mechanisms in both prokaryotic and eukaryotic

Int. J. Mol. Sci. **2011**, *12*, 6566-6596; doi:10.3390/ijms12106566

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ISSN 1422-0067
www.mdpi.com/journal/ijms

Review

The Potential of Antimicrobial Peptides as Biocides

Garry Laverty [1,*], Sean P. Gorman [2] and Brendan F. Gilmore [2]

Research article
Journal of PeptideScience

Received: 13 May 2015 Revised: 26 June 2015 Accepted: 6 July 2015 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/psc.2805

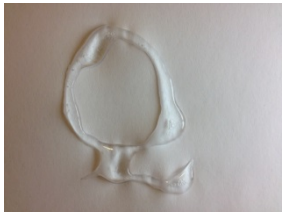
Anti-biofilm activity of ultrashort cinnamic acid peptide derivatives against medical device-related pathogens

Garry Laverty,* Alice P. McCloskey, Sean P. Gorman and Brendan F. Gilmore

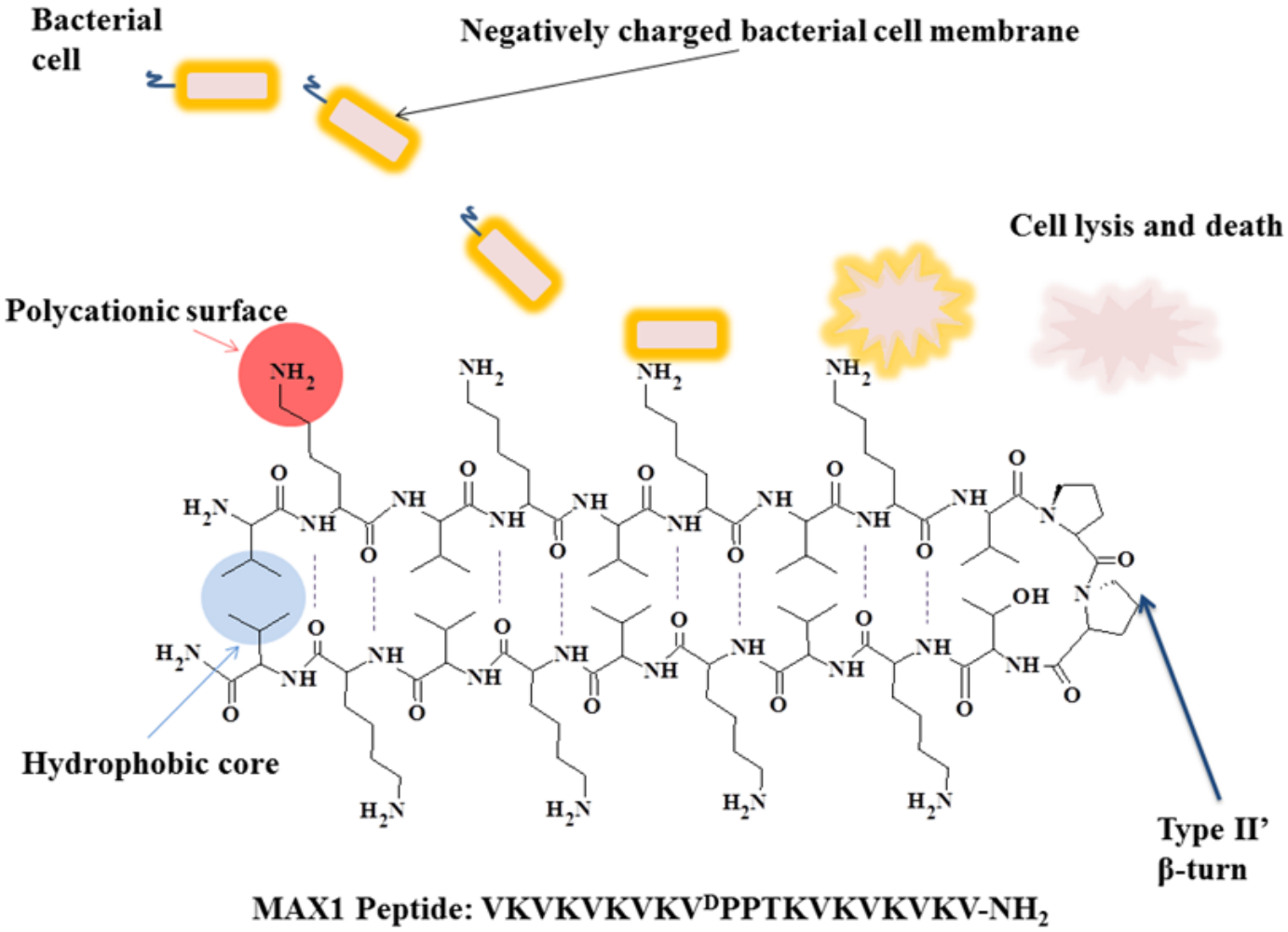
Rational Design of Antimicrobial Peptide Motif vs Self-assembly

Antimicrobial Activity	Propensity to Self-assembled hydrogels
Hydrophobic/Hydrophilic (Charge) ratio (more important with regard to antimicrobial activity than size)	**Hydrophobic/Hydrophilic balance**
Interactions with microbial extracelluar membranes	Non Covalent intermolecular interactions (e.g. Van der Waal's, π-π stacking)
Interaction with intracellular targets/processes (DNA, RNA, enzymes, protein synthesis)	Ability of peptide to form hydrogen bonds with each other and with water

McCloskey A.P., Gilmore, B.F., Laverty, G. (2014) Evolution of Antimicrobial Peptides to Self-Assembled Peptides for Biomaterial Applications. *Pathogens*. *3*(4); 791-821.



Microbiological Applications



- McCloskey A.P., Gilmore, B.F., Laverty, G. (2014) Evolution of Antimicrobial Peptides to Self-Assembled Peptides for Biomaterial Applications. *Pathogens*. *3*(4); 791-821.
- Schneider, J. P.; Pochan, D. J.; Ozbas, B.; Rajagopal, K.; Pakstis, L.; Kretsinger, J. (2002) Responsive hydrogels from the intramolecular folding and self-assembly of a designed peptide. J. Am. Chem. Soc., 124, 15030-15037.

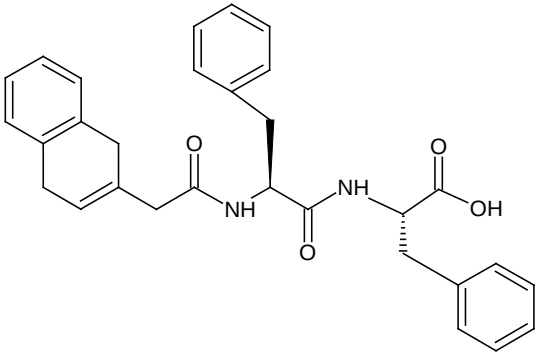
Antimicrobial Force Fields: Translate from Science Fiction to Science FACT

Protecting surfaces from pathogenic microorganisms: adherence and biofilm formation

Self-assembled Ultrashort Peptide Gels

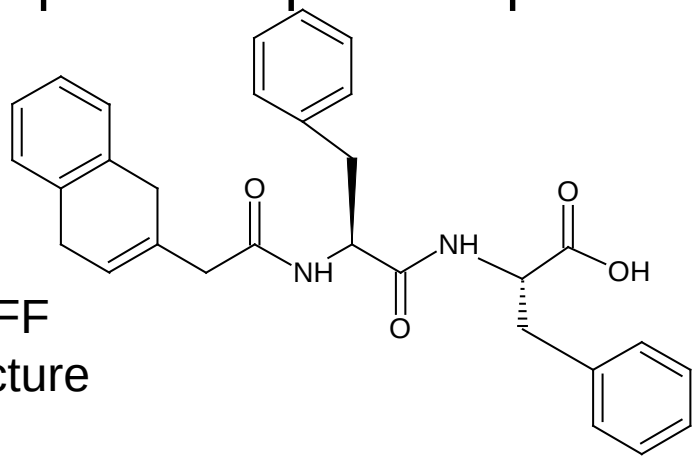
- 2013 Research Placement Prof. Bing Xu Lab, School of Chemistry, Brandeis, Waltham, Boston, USA
- Successful in producing a series of ultrashort peptides (< 7 amino acids) that self-assembled at physiological pH
- (X_1-FF-X_2)
- More cost effective
- Hydrophobicity provided by inclusion of a naphthalene (Nap) grouping (at X_1 position) and varying quantity of phenylalanine in primary structure

NapFF structure

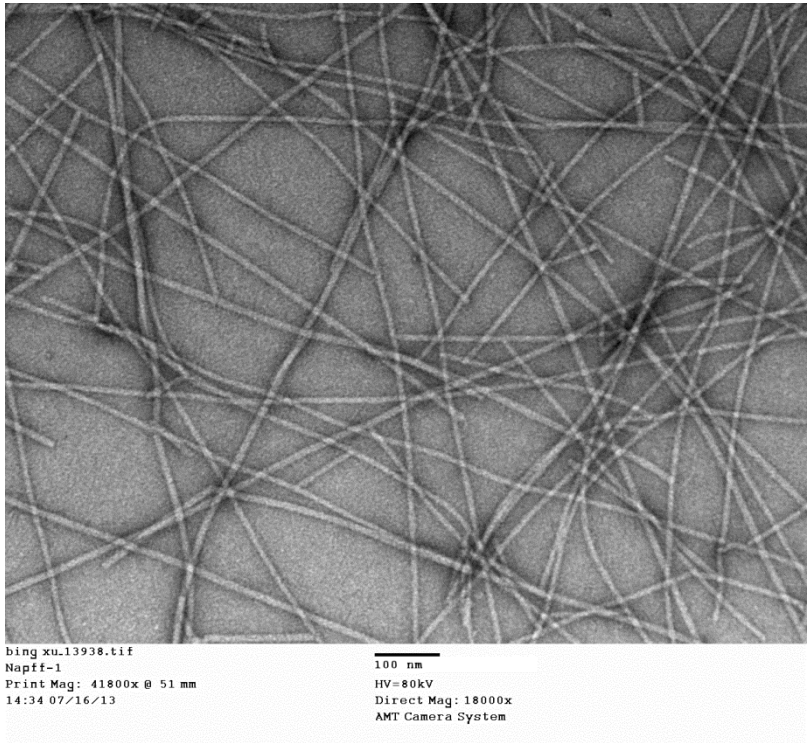


Preparation of Ultrashort Self-assembled Peptide Gels

- Nanofibre structures formed
- Due to π-π interactions between aromatic moieties
- Change physiochemical environment e.g. pH (pK_a), enzymes, temperature, light
- Beta-sheets
- Cytocompatible: up to 200μM



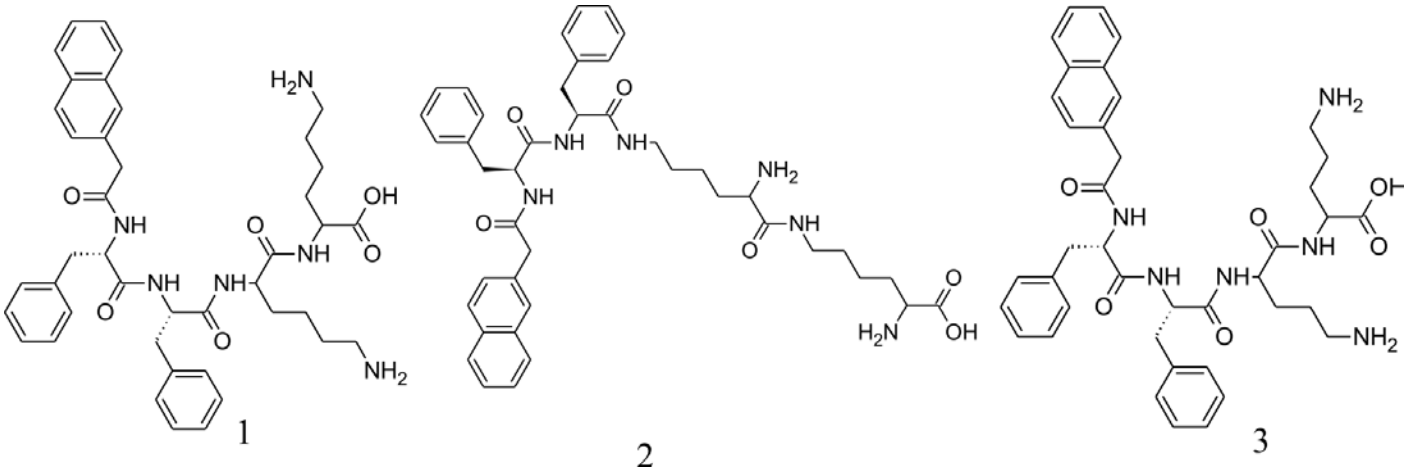
NapFF structure



TEM of 1%w/v NapFF showing nanofibre structures.

Ultrashort Cationic Variants: Primary Structures

- Charge: Inclusion of cationic amino acids
  - 1) Lysine
  - 2) Ornithine
  - 3) epsilon (ε) Lysine
- Minimum of 2 charged units required for antimicrobial activity
- Primary amine group provides cationic charge
- Cationic amino acids vary by number of methylene units on R-group: alters pKa and cationicity of R-group also



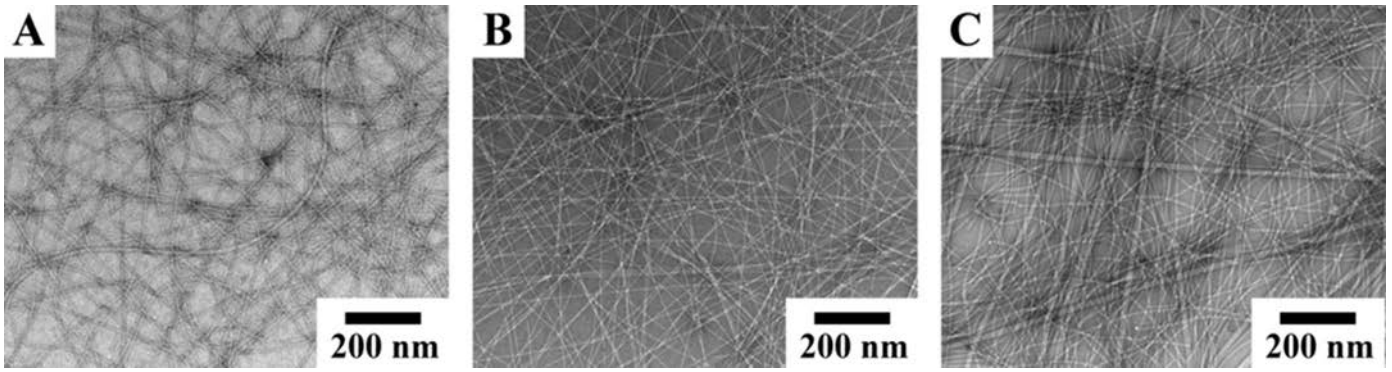
-Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). Ultrashort Cationic Naphthalene derived Self-assembled Peptides as Antimicrobial Nanomaterials. *Biomacromolecules*; 15: 3429−3439.

-Laverty, G., Gorman, S.P. and Gilmore, B.F (2012). The Adherence of *Staphylococcus epidermidis* to Antimicrobial Peptide Incorporated poly(2-hydroxyethyl methacrylate) Hydrogels. *Journal of Biomedical Materials Research: Part A* 100A; 1803–1814.

Ultrashort Cationic Variants: Self-assembly

- Form Self supporting hydrogels at pH 7.4: pH triggering method

Optical images of gel (A) NapFFOO, (B) NapFFKK, (C) NapFFεKεK, at a concentration of 1% w/v and pH of 7.4 in water

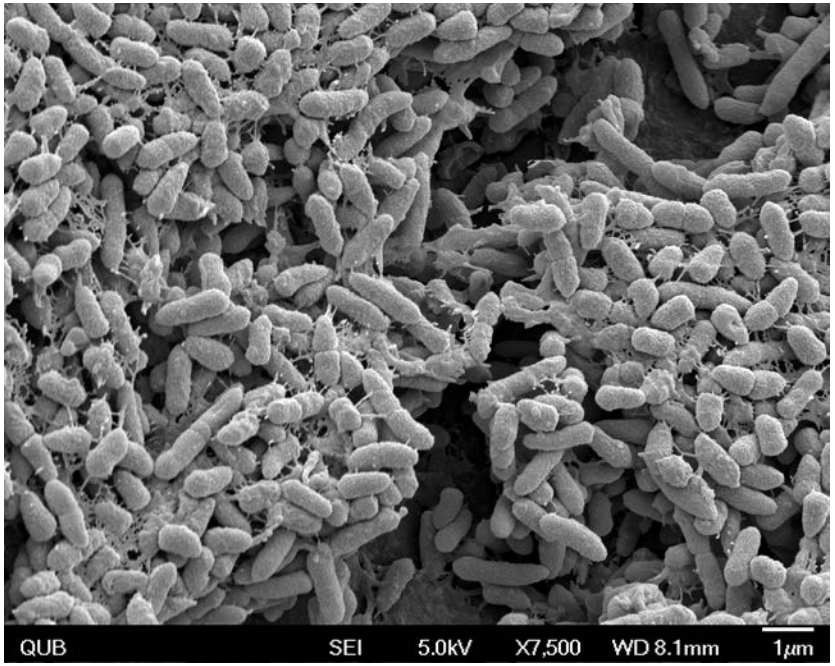


Transmission electron microscopy (TEM) images of (A) NapFFOO, (B) NapFFKK, (C) NapFFεKεK, at a concentration of 1% w/v and pH of 7.4 in water

Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). Ultrashort Cationic Naphthalene derived Self-assembled Peptides as Antimicrobial Nanomaterials. *Biomacromolecules*; 15: 3429−3439.

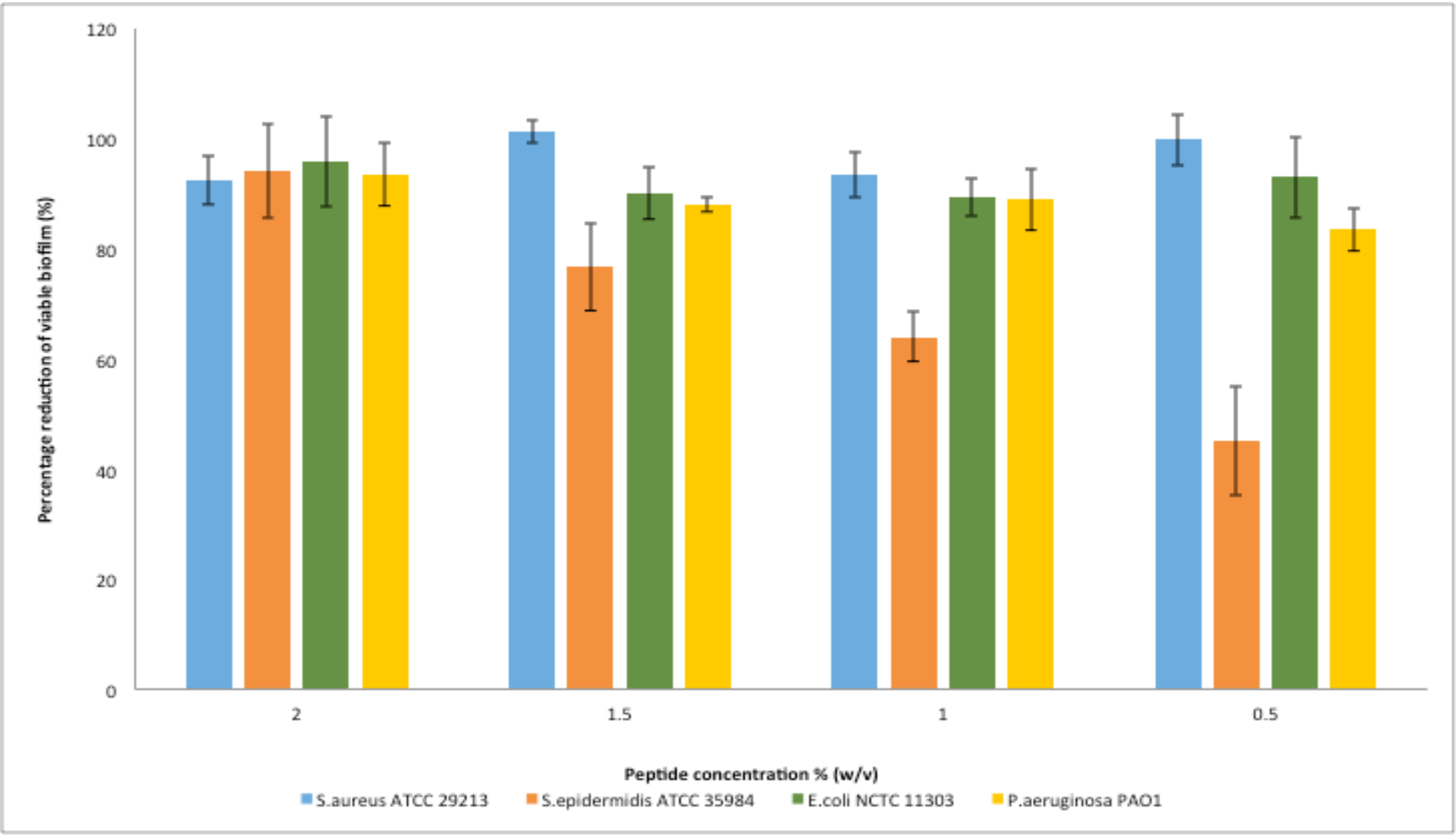
Anti-Biofilm Activity

- alamarBlue Cell Viability assay: 24 hour grown Biofilms
- Gram-positive
  - *Staphylococcus epidermidis* (ATCC 35984)
  - *Staphylococcus aureus* (ATCC 29213)
- Gram-negative
  - *Pseudomonas aeruginosa* (PAO1)
  - *Escherichia coli* (NCTC 11303)
- Medical device related pathogens

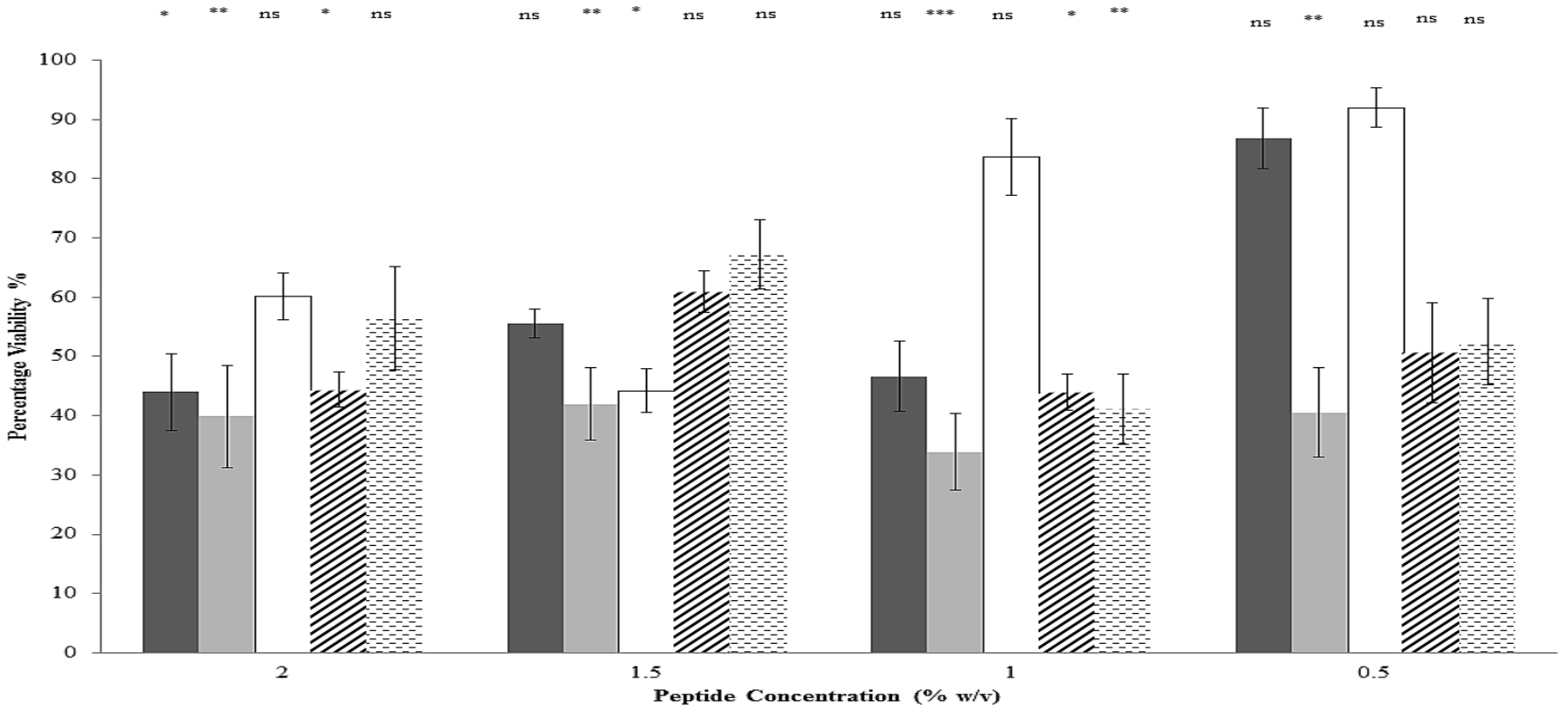


SEM *Pseudomonas aeruginosa* (PAO1) attached to catheter surface

NapFFKK: alamarBlue Biofilm Viability Assay: 24 hours



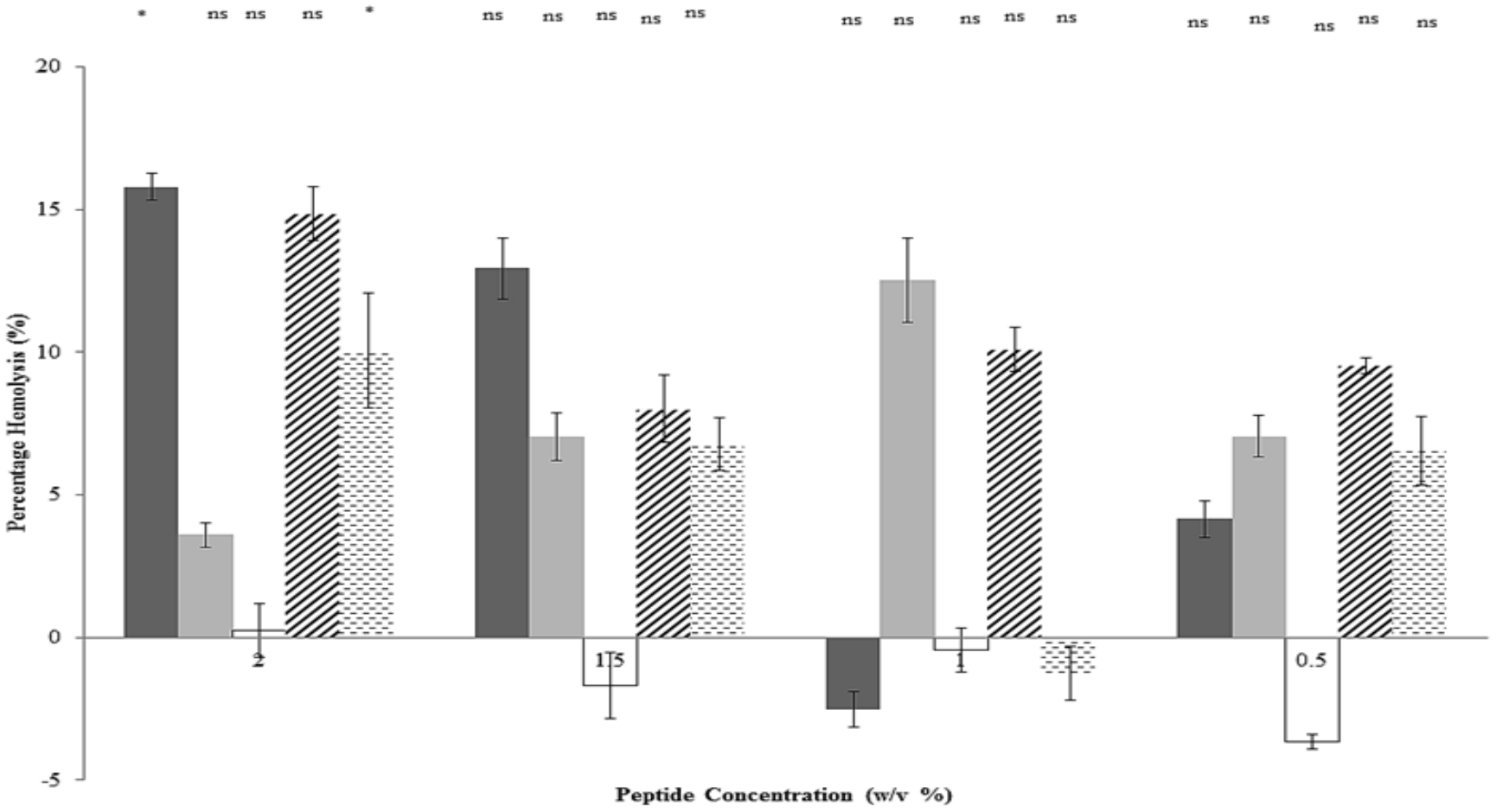
Toxicity: Tissue Culture & Haemolysis



Percentage viability of CCL 1 [NCTC clone 929]- murine fibroblasts subcutaneous connective tissue monolayer cells after 24 hour exposure to naphthalene derived self-assembled hydrogels utilizing an alamarBlue assay. Results are displayed as a mean of 8 replicates

Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). *Biomacromolecules*; 15: 3429−3439.

Haemolysis: 1 hour



Percentage hemolysis of the naphthalene peptides against equine erythrocytes. Each value is expressed as the mean of six replicates, incubated at 37 °C for 1 hour

Global Impact! August 2014

Belfast Telegraph

Antibacterial gel from Queen's University Belfast scientists can kill the toughest of hospital superbugs

RTÉ News

Scientists in Belfast develop new gel to fight superbugs

abc30 ACTION NEWS KFSN-TV • FRESNO

SECTIONS TRAFFIC VIDEO Fresno County North Valley

HEALTH WATCH

ATTACKING SUPERBUGS IN THE HOSPITAL

More than 70,000 Americans will die each year from an infection they caught in the hospital. Superbugs have invaded hospitals and proved difficult to treat.

BBC

Queen's University Belfast scientists create 'superbug gel'

UTV

Gel developed to tackle superbugs

THE IRISH TIMES

Queen's University scientists take fight to hospital superbugs

DAILY NEWS NEW YORK

Gel that wards off superbugs may be coming soon

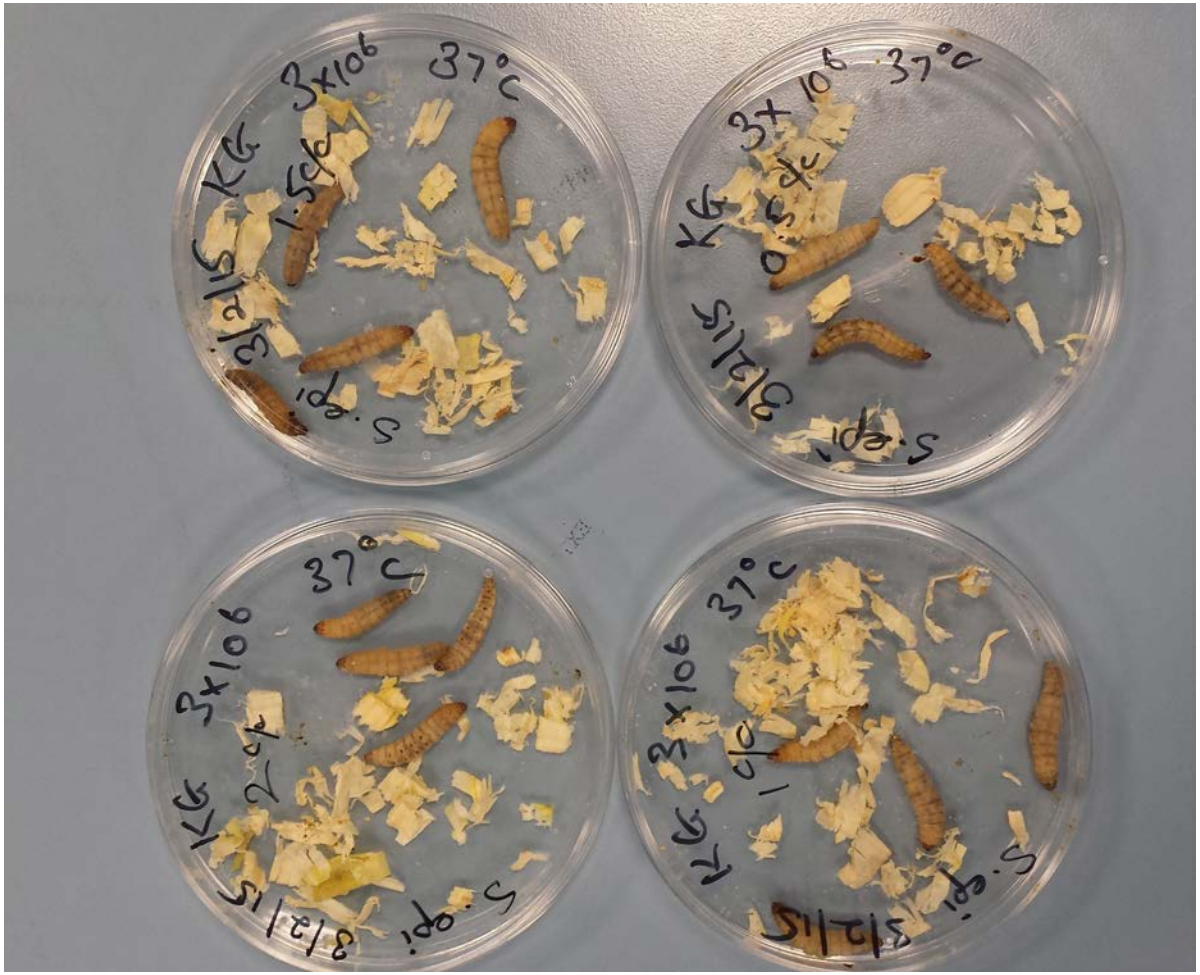
WTOP 103.5 FM

Washington, DC

New research shows promise in preventing common superbug infections

Galleria mellonella (Waxworm) assay

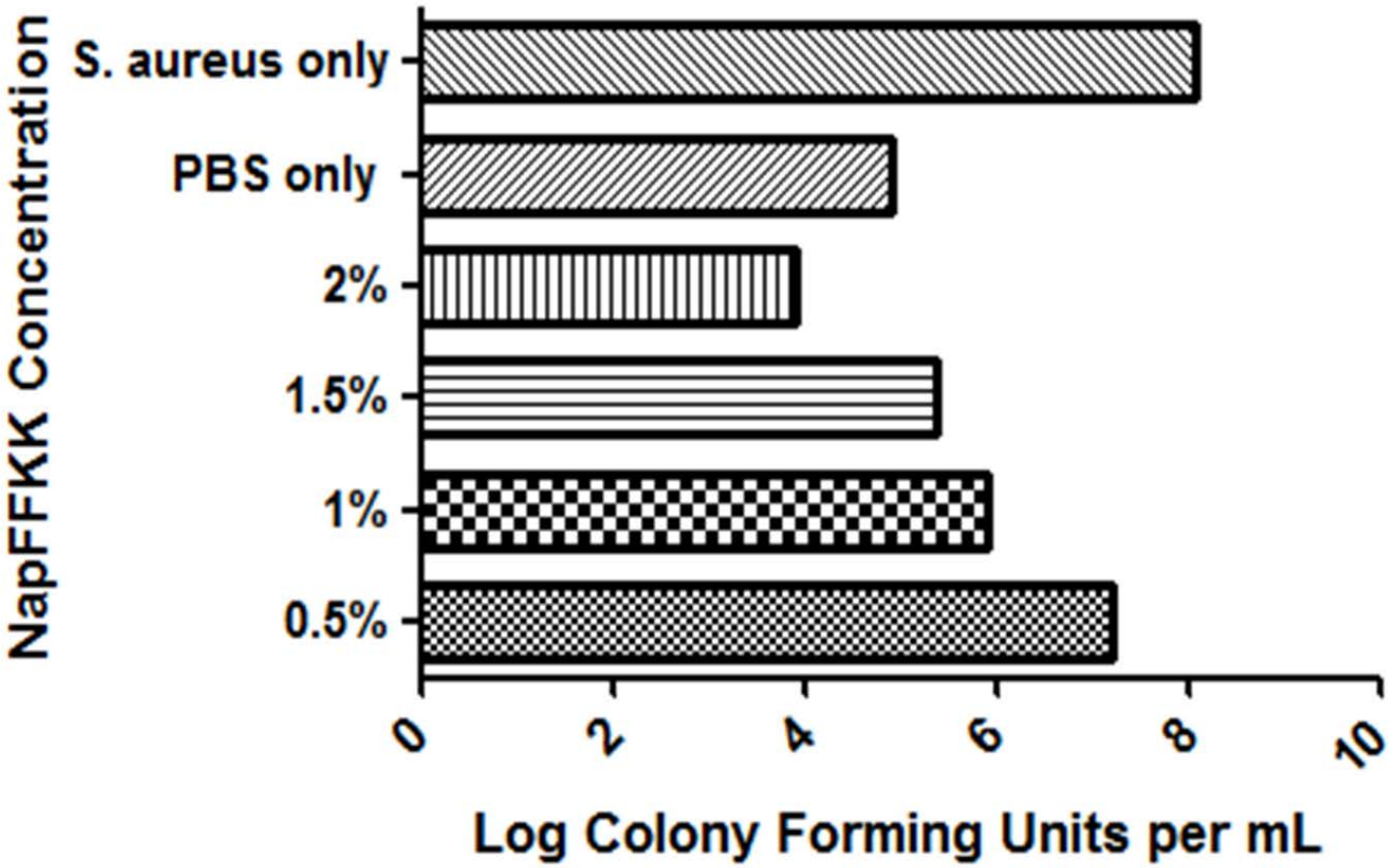
National Centre for the Replacement Refinement & Reduction of Animals in Research



Non viable *Galleria mellonella*

Data demonstrates biocompatibility (NapFFKK) and reduction in bacterial load with *S.aureus* (ATCC 29213), *S.epidermidis* (ATCC 35984), *E.coli* (NCTC 11303) and *P. aeruginosa* (PAO1)

G.mellonella assay: *S.aureus*

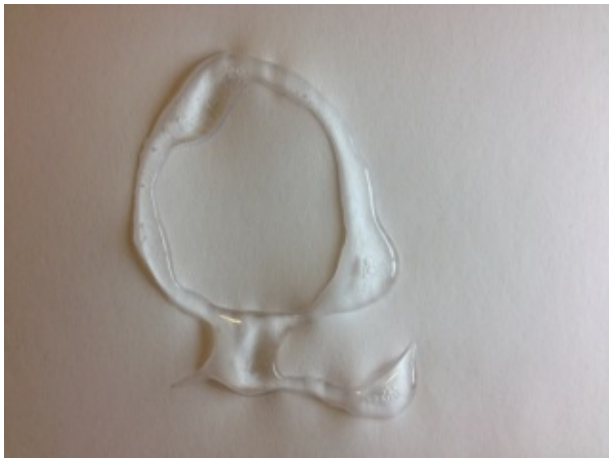


Bacterial counts (Log CFU/mL) from haemolymph extracted 72 hour following inoculation with 20µL of 1x 10^5 *S.aureus* and treatment 2 hour later with 20µL NapFFKK.

McCloskey, A.P., Lee, M., Gilmore, B.F., Laverty, G*. *Galleria mellonella* as an *in vivo* infection model: the treatment of medical device related pathogens by Ultrashort Self-assembled Peptides. *In Preparation*

Conclusions/Future Perspectives

- Greater selectivity was shown against biofilm bacteria compared with mammalian cells: aim to improve!
- NapFFKK shows particular promise
- Addition of alternative functionalities extends activity (e.g. anti-inflammatory/anticancer)
- Further research is required:
  - Is antimicrobial activity linked to gelation/self-assembly?
  - Gelation and antimicrobial activity in response to the infectious stimuli *in vivo*
    - *pH*
    - *Enzymes*
  - Biostability assays
  - Selective for specific resistant isolates



Future Applications within Microbiology

- Conjugate to existing biomaterial polymers (Atomic Transfer Radical Polymerization)
- Wound dressings (Injectable)
- Tissue Scaffolds
- Drug Delivery (e.g. Lung infections) and as a vehicle for delivery of other drugs

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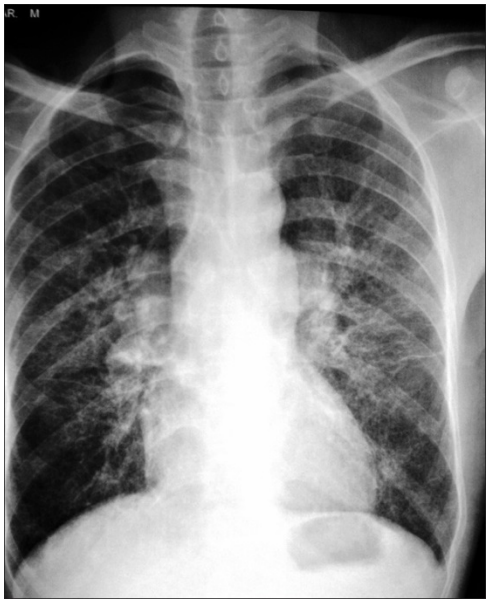

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- Alice McCloskey (DEL funded PhD student)
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- Lucia Murias (Visiting Research Assistant)
- Sophie Gilmore (S*f*am: Students into Work)
- Merissa Lee (S*f*am: Students into Work)

Research Group Website:
http://lavertylab.weebly.com

- The Xu Group, School of Chemistry, Brandeis.